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Review: Fetal-maternal communication via extracellular vesicles – Implications for complications of pregnancies

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ABSTRACT

The maternal physiology experiences numerous changes during pregnancy which are essential in controlling and maintaining maternal metabolic adaptations and fetal development. The human placenta is an organ that serves as the primary interface between the maternal and fetal circulation, thereby supplying the fetus with nutrients, blood and oxygen through the umbilical cord. During gestation, the placenta continuously releases several molecules into maternal circulation, including hormones, proteins, RNA and DNA. Interestingly, the presence of extracellular vesicles (EVs) of placental origin has been identified in maternal circulation across gestation. EVs can be categorised according to their size and/or origin into microvesicles (~150–1000 nm) and exosomes (~40–120 nm). Microvesicles are released by budding from the plasmatic membrane, whereas exosome release is by fusion of multivesicular bodies with the plasmatic membrane. Exosomes released from placental cells have been found to be regulated by oxygen tension and glucose concentration. Furthermore, maternal exosomes have the ability to stimulate cytokine release from endothelial cells. In this review, we will discuss the role of EVs during fetal-maternal communication during gestation with a special emphasis on exosomes.

Contents

1. Introduction

The human placenta is a unique, transient organ that ensues its development with the implantation of the blastocyst in the uterine wall [1]. Throughout pregnancy, it provides nutrition, gas exchange, waste removal, a source of haematopoietic stem cells and

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endocrine and immune support for the developing fetus, as well as the point of molecular exchange between the maternal and fetal systems [2].

Functionally, the human placenta is a villous tree covered with an abundance of trophoblast cells that can be classified as cytotrophoblast (CT), extravillous trophoblasts (EVTs) and syncytiotrophoblasts. These cells have specific functions during gestation [3], where syncytiotrophoblasts are in direct contact with the maternal circulation for nutrients [4]. The human placenta can release a wide range of molecules which enable the maternal physiology to accommodate fetal requirements during gestation. Interestingly, it has been described that the placenta can also communicate with the maternal physiology via extracellular vesicles (EVs) [5–7].

An increase in the release of EVs into maternal circulation as the pregnancy progresses has been noted in both healthy and pathological pregnancies, GDM, and PE [6–9]. Interestingly, the presence of exosomes has been identified in maternal plasma as early as 6 weeks of gestational age [9]. These vesicles encapsulate a diverse cargo of proteins, lipids and nucleic acids that are constitutively released into the maternal circulation. They are subsequently taken up by cells of the maternal immune and vascular systems, hence modulating the overall maternal physiological system to adapt to pregnancy-induced changes [6]. Nevertheless, in complicated pregnancies, this mode of cell signalling plays a role in the manifestation of physical symptoms of disease states. This is particularly because the release of the extracellular vesicles is dependent upon the microenvironment that they are exposed to [10].

This review will discuss the current body of knowledge on EVs during gestation with emphasis on the trafficking of placental vesicles into maternal circulation to regulate immune and metabolic adaptations to pregnancy. However, there is a gap in the literature pertaining to the standardisation of isolation methods to enrich specific populations of EVs, such as exosomes.

2. General characteristics of extracellular vesicles

A growing amount of evidence has emerged identifying EVs as a form of intercellular communication [11]. EVs (50 nm-2um) are lipid-bilayer structures released from cells into the extracellular environment. They contain an array of proteins, lipids, RNAs and DNA. They are released from several cell types, such as trophoblasts [12], erythrocytes [13] and endothelial cells [14]. Initially thought to be cellular 'debris', EVs were later observed to interact with and modulate the bioactivity of specifically targeted cells [15]. Recent research has elucidated that EVs also modulate key processes in pregnancy, including regulation of immune responses, migration/invasion of placental cells, and cellular adaptations to the physiological changes underlying gestation [16].

EVs comprise several different types of vesicles. Two major groups of interest are exosomes and microvesicles, which can be further distinguished by size, biogenesis and content [11]. Exosomes (40–120 nm) are bioactive membrane nanovesicles formed from the endocytic pathway. The inward budding of the plasma membrane results in the formation of multivesicular bodies, which subsequently internalise to form intraluminal vesicles. These intraluminal vesicles are then released into the extracellular environment via exocytosis as exosomes. As a result of their unique biogenesis, exosomes are enriched with proteins such as CD63, CD9, CD81. Furthermore, they contain a variety of internal markers such as Tumour Susceptibility Gene 101 (TSG101) and Apoptosis-linked gene 2-interacting protein X (ALIX). Exosomes can also be identified by distinct cup-shaped morphology and a buoyant density in a sucrose gradient ranging from 1.13 to 1.19 g/mL. On the other hand, microvesicles (MVs) (approximately 0.2-1 μm), which are the most heterogeneous population, are secreted from the plasma membrane through direct budding or shedding of the membrane in response to cellular activation or stress [17]. A wide range of EVs from placenta origin has been dentified in maternal circulation under both normal and pathological conditions.

3. Placental vesicles in maternal circulation

Exosomes and microvesicles are released under normal and pathological conditions, with more potent effects on the physiology of target cells than single-molecule mediators such as lipids, hormones or cytokines. Placental vesicles have been identified in maternal circulation across gestation [7]. Notably, the secretion of vesicles was found to be increased during pregnancies complicated by gestational diabetes [7] and preeclampsia [18]. Moreover, EV release during pregnancy is modulated by particular features of the cellular microenvironment such as low oxygen tension (*i.e.* hypoxia) or high glucose concentration [19].

The concentration of EVs in circulation is higher in pregnant women compared to non-pregnant women [7,20]. This increased concentration may be attributed to the population of placental exosomes, which have been identified in maternal circulation at early gestation (~6 weeks) [9]. EVs may play key roles in shaping the complex physiological changes during pregnancy, including establishment of the fetal-maternal circulation. This process begins with EVT invasion and is complete within 10 weeks of gestation. The invasion of EVTs is crucial for the remodelling of the spiral arteries (SpA), as well as preventing contact with the maternal blood flow in the intervillous space. Establishment of the maternal blood flow towards the end of the first trimester occurs when cytotrophoblasts fuse to form when cytotrophoblasts fuse to form layers of multinucleated syncytiotrophoblasts. These layers are bathed in soluble proteins and nutrients and cover the majority of the surface of the placenta [21]. The maternal blood then has direct contact with EVTs and syncytiotrophoblasts, becoming the dominant site for the release of EVs into the maternal circulation.

The release of EVs by EVTs in early pregnancy has been demonstrated through the detection of soluble proteins such as human leukocyte antigen (HLA-G) [22]. HLA-G is only expressed in EVTs, where HLA-G^+-EVs have been detected in the maternal circulation throughout pregnancy decreasing with term [23]. The EVs released from EVT using the Swan71 cell line revealed that they can release exosomes [24]. The findings of this study, as well as the detection of HLA-G^+ -EVs, strongly indicates that EVTs release both exosomes and microvesciles.

The regulation and release of EVs are dependent on the microenvironment, where composition and concentration have been reported to be altered in the plasma of pregnant women compared to non-pregnant women [25]. Knight et al. [26] found that consistent with placental origin, increased levels of placenta-derived EVs have been detected in the uterine vein blood compared to the peripheral blood [26]. When comparing pregnant and non-pregnant women, the concentration of exosomes was found to be approximately 50-fold greater. This concentration increases with disease severity and oxidative stress [7]. Interestingly, it has been found that the concentration of EVs in circulation of preeclampic women is approximately 40% greater than that of healthy, pregnant women. This suggests that EVs may be implicated in exacerbations of the maternal systemic innate immune system and vascular dysfunction [26].

Moreover, levels of placental-alkaline phosphatase-positive ($PLAP^+$)-EVs were detected in the maternal circulation throughout pregnancy [26]. These $PLAP^+$-EVs have been isolated directly from the plasma of pregnant women at 26 and 28 weeks of gestation [20], where they continuously increase during the first 12 weeks of

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